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Review paper

FOODBORNE VIRUSES — AN EMERGING PATHOGENS

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Abstract
Viral foodborne illnesses which have become a significant cause of all reported foodborne illnesses in recent years and considered as an emerging risk in veterinary public health. Foodborne transmission can occur by contamination of food by infected food handlers, by contamination of food during the production process and by consumption of products of animal origin harboring a zoonotic virus. Microbiological genomics studies discovered that noroviruses and hepatitis A viruses were primarily associated with food-handler transmission and sewage-contaminated foods while hepatitis E was associated with consumption of raw or undercooked meat of pig or wild animals. Routine harmonized surveillance of viral outbreaks, and surveillance of virus occurrence in food commodities, in combination with systematic strain typing, and joint expertise from veterinary, food, and clinical microbiologists would be recommended to aid source attribution studies and identify risk prevention measures.

Introduction

Over the last decades, due to rise of discretionary incomes in Europe and North America, increased urbanization and altered eating habits, worldwide food industry has changed from being locally oriented and supply-driven to become globalized and demand-driven. In order to satisfy consumer groups demanding safety, fair trade, "green" production, and animal welfare-related changes in production practices, policy makers imposed high hygienic standards and various control strategies for pathogenic bacteria, viruses, and parasites. These measures primarily concerned with common causes of food-borne diseases such as unsafe raw food, abused temperature, poor storage infrastructures, inadequate cooking, poor personal hygiene, improper handling methods, and cross-contamination of cooked food with uncooked raw food. Although contamination prevention and control strategies are mostly successful in reduction of food-borne diseases, they also demonstrate the weakness of the global food supply: if there is a fault in the process, then contamination may occur with pathogens from across the globe, including those that have recently emerged [1]. This proved to be challenging for diverse and complex food systems, especially those in less-developed countries.

In this paper we will address viral foodborne illnesses which have become a significant cause of all reported foodborne illnesses in recent years and considered as an emerging risk in veterinary public health.

Food-borne viruses and their role in food safety

Currently known viruses that can infect humans are grouped into 22 families. In addition to this, the recent advances in molecular techniques that allow characterization of all genetic material in a given sample has led to the identification of several new viruses in recent years, most of which remain to be fully characterized [2, 3]. Viruses are strict intracellular parasites and cannot replicate outside a specific living cell. The host cell treats viral genetic information as if it were its own. Replication of viruses occurs by transcription and translation of the viral genome using host cell mechanisms. It is not possible to culture them in an environment free of living cells, and therefore the number of viral particles does not increase in food and water during production, processing, transport, and storing. Sensory characteristics of products containing these pathogens and those of non-contaminated food are identical [4, 5]. Transmission of the virus does not only depend on its interaction with the host, but also on the influence of the external environment. Outside the host organism, viruses are inert particles without their own metabolism. The longer they survive in the infectious state environment, the higher is the probability of transmission and spread of infection [6].

Foodborne transmission has been documented for viruses belonging to at least 10 taxonomic families, and the diseases associated with these infections range from mild diarrheal illness to severe encephalitis. Foodborne transmission can occur by:

- contamination of food by infected food handlers (frequently),
- by contamination of food during the production process (frequently — in bivalve molluscan shellfish or berry fruit production),
- by consumption of products of animal origin harboring a zoonotic virus (very rare).

The first and second mean of transmission applies to viruses that are transmitted by the faecal-oral route, hence infect their host after ingestion, followed by invasion of cells in the gut epithelium, and subsequent replication in the same site or elsewhere in the body. WHO and FAO [7] have ranked noroviruses (NoV), group A rotaviruses, and hepatitis A viruses (HAV) as priority hazards. When it comes to emerging hazards, hepatitis E virus (HEV), Nipah viruses, H5N1 avian influenza viruses and SARS

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coronavirus were considered to be of greatest importance. They have also linked risk of specific virus to a specific commodity, and the importance of that commodity in causing viral foodborne illness and found following virus-commodity combinations for which prevention and control measures should be thoroughly considered:

- NoV and HAV in bivalve molluscan shellfish;
- NoV and HAV A in fresh produce;
- NoV and HAV in prepared foods;
- Rotaviruses in water for food preparation;
- Emerging viruses in selected commodities.

Foodborne NoV outbreaks are often linked to food handlers who infect foods that are eaten raw or not further processed (ready to eat (RTE) foods) prior to consumption [8]. In many of these outbreaks, a sick food-handler or food-handler with a recent history of gastroenteritis was noticed. Workers have often been in contact with ill family members including children before the worker handled food. The most common food worker errors identified in relation to outbreak of NoV and HAV are food handling by an infected person or carrier of virus together with bare-hand contact by handler (RTE foods) and failure to properly wash hands (9). Poor personal hygiene was also identified as a contributing factor in outbreaks with NoV assigned as the causative agent. Food handlers can contaminate food either with particles from vomit (NoV) or from faeces (NoV/HAV) when employing insufficient personal hygiene after using toilets. Asymptomatic food workers are implicated more frequently than symptomatic workers, which helps explain the difficulty in detecting and stopping an outbreak by excluding ill food workers [9].

Food contamination at site happens when food is contaminated during the primary production of risky commodities, such as berries, green vegetables or bivalve molluscan shellfish. In these cases sewage or wastewater contamination are the primary source of food-borne viruses, and NoV and HAV were considered to be priority concerns according to aforementioned WHO/FAO opinion [7]. Sewage or contaminated water frequently contain multiple RNA viruses, opposite to cases in which food handler contamination occurred. In this case, cohabitation of different (ss+) RNA viruses and subsequent co-infection of a human cells by genetically distinct viral strains can lead to the generation of recombinant viruses shuffling their individual mutations and thus making unpredictable effects on viral behavior and virulence.

Zoonotic food-borne infection occurs when meat, organs, or other products from an infected animal are consumed [10]. For viruses, this is the very rare mode of transmission, although in every emerging disease outbreak this should be investigated. This is especially case with hepatitis E virus since infected pig liver (of both domestic pig and wild boar) consumed raw or undercooked is the main source of infection/contamination. Also, severe acute respiratory syndrome (SARS) and Nipah virus have been transmitted through food-related incidents [11, 12].

Common foodborne viruses

Norovirus

NoV belong to the Family *Caliciviridae*, that is divided into five genera. NoV and Sapovirus are the two genera of the family *Caliciviridae* that contain viruses that cause infections in humans. NoV have also been detected in pigs, cattle, mice, cats, dogs, and sheep, and sapoviruses in pigs. The other genera of the family Caliciviridae are Lagovirus, Vesivirus, and Nebovirus encompassing viruses infecting rabbits, and brown hares (lagoviruses), sea lions, swine, cats, dogs, fish, seals, other marine animals, cattle and primates (vesiviruses), and cattle (Nebovirus) [13]. In humans, NoV infection typically causes acute gastroenteritis, with the most common symptoms being nausea, vomiting, diarrhea, and stomach pain. Symptoms usually develop 12 to 48 hours after infection. The disease normally lasts between 1 and 3 days.

NoV can be divided into five distinct genogroups, based on phylogenetic analyses of the capsid protein (GI-GV). Viruses of GI, GII and GIV are known to infect humans. GII viruses have additionally been detected in pigs, and GIV viruses have been detected in a lion cub and a dog. GIII viruses infect cattle and sheep and GV viruses infect mice. Recombination between viruses from different genogroups is rare suggesting that this constitutes a species level in taxonomy. Within each genogroup, viruses are further divided into genotypes [14].

NoV illness prevalence is highest in young children (< 5 years) and the elderly [15]. Factors that contribute to the significant impact of noroviruses include a large human reservoir, low infection dose (only 10 to 100 viral particles), their environmental robustness, the short-lived immunity to noroviruses (18 months at most), and the ability to be transmitted by various routes. Majority of incriminated foods includes shellfish which feed by filtration of surrounding water, then berry fruit and green vegetables contaminated during soil fertilizing shortly before picking or watered by contaminated municipal water [5, 16, 17].

Most NoVs can also escape the receptor-blocking activities from antibodies triggered by earlier infections due to accumulated mutations in genome [18, 19]. Viruses are present in faeces and vomitus of diseased people at extremely high levels, up to 10^{10} viral particles per gram of stool [20]. The major obstacle to research human noroviruses has been the lack of a robust and reproducible in vitro cultivation system. Such a system is critical to achieve a full mechanistic understanding of human noroviruses replication, stability, evolution and pathogenesis. However, recently stem-cell derived, non-transformed human intestinal enteroid (HIE's) cultures validated as an appropriate pre-clinical model for clinically important enteric infections have been reported [21].

When it comes to prevalence, WHO estimates that Norovirus is the most common cause of foodborne illness in the European region with close to 15 million cases each year, causing more than 400 deaths. In the Netherlands,

Norovirus remains the key pathogen causing food-related outbreaks in 2016 as in previous years, followed by *Salmonella* and *Campylobacter* [22].

EFSA, ECDC and FVO have been systematically monitoring whole picture of the state of affairs concerning the Norovirus issue. European Union-coordinated monitoring program on the prevalence of norovirus in raw oysters was initiated. The objective of the study was to estimate the European prevalence of norovirus-contaminated oysters at production areas and batches of oysters at dispatch centers, with a 95% level of confidence and a level of precision of 5% considering an expected prevalence of 50%. The survey started in November 2016 and finishes in October 2018 [23]. The EFSA delivered a scientific opinion on the evaluation of heat treatments, different from those currently established in the EU legislation that could be applied to live bivalve molluscs. Of particular relevance are the achievement of at least 90 °C for at least 90 s in the molluscs flesh and the inactivation of viruses [24].

Currently, EU regulatory authorities are focusing in following areas in Norovirus combat: (i) whole genome sequencing for the characterization of Norovirus and other foodborne viruses; (ii) surveillance to generate more information about levels of Norovirus occurring in food; (iii) refinements to current RT-PCR to improve detection of low numbers of norovirus particles in all food matrices; (iv) the binding properties and possible methods of inactivation of norovirus; (v) the effectiveness of depuration (or alternatives such as high pressure, UV, ozone, irradiation) in removing norovirus from oysters; and (vi) establishment of the infectious dose in different food commodities including shellfish and fresh produce (lettuce and berries).

Hepatitis A virus

Hepatitis A is caused by the hepatitis A virus (HAV) which belongs to genus Hepatovirus within family *Picornaviridae*. Hepatoviruses have only been found in humans and primates, suggesting there is no introduction from any other reservoir. Based on genetic diversity, hepatitis A viruses are divided into six lineages or genotypes, of which genotypes I–III infect humans [25]. It consists of a non-enveloped icosaedral capsid of around 30 nm in diameter containing a positive ssRNA genomic molecule of 7.5 Kb [26]. HAV is a unique picornavirus because it does not inhibit host-cell protein synthesis to allow a regulated ribosome traffic rate thus ensuring the proper protein folding [27]. Capsid folding is critical to permit a long period-environmental stability for a virus transmitted through the faecal-oral.

Since proper sanitation and good hygienic conditions greatly reduce transmission rate of HAV its prevalence is significantly lower than prevalence of NoVs, [28]. In highly endemic regions, HAV is one of the childhood infections that, in the majority of cases, runs an asymptomatic course, while triggering a protective immune response that lasts long, possibly even lifelong [29]. HAV is quite stable outside a host and, therefore, can persist on contaminated environments, food, and water for a quite long time. Food- and water-borne outbreaks have been documented, although again, as for NoVs, the most common mode of transmission occurs between persons. Incidence risk of food-borne HAV at present comes from introduction through food into regions where population immunity is relatively limited. Foods commodities susceptible to contamination during the production phase, such as bivalve filter-feeding oysters, clams, mussels or commodities that are irrigated with water that may be contaminated (lettuce, green onions, and soft fruits, such as raspberries and strawberries). These foods should be considered the principal targets for virological analysis. Nevertheless, in roughly 40% of the reported cases of hepatitis A the source of infection cannot be identified [30]. The first documented shellfish-borne outbreak of "infectious hepatitis" occurred in Sweden in 1955, when 629 cases were associated with raw oyster consumption. However, the most significant outbreak of HAV infection occurred in Shanghai, China, in 1988, in which almost 300,000 cases were caused by consumption of clams harvested from a sewage-polluted area. A specific problem with shellfish is that the current microbiological quality control criteria are based on quantitative testing for *E. coli* contamination, which often fails to predict the presence or absence of viruses. Water-depurated shellfish have been associated with outbreaks of norovirus, hepatitis A, gastroenteritis, and other viral diseases [31].

Hepatitis E virus

HEV is a non-enveloped icosahedral virus with a diameter of 35 nm, classified into the unassigned genus Hepevirus. The genome consists of one positively oriented single-stranded RNA molecule and around 7 kb in length. The major ORFs are ORF-1, which encodes a non-structural polyprotein, ORF-2 encoding the capsid protein and ORF-3 encoding a phosphoprotein.

The HEV strains can be grouped into 4 genotypes, with different spatiotemporal distribution and different host. Genotypes 1 and 2 have been found solely in humans, i. e. genotype 1 is endemic in Asia and Africa where inhabitants are exposed to the virus due to poor sanitary conditions and sewage overspill that results from heavy rainfall [32]. In these conditions surface water is contaminated that is used for drinking water production or as source for water used for household tasks, so this explains the magnitude of outbreak. Genotype 2 is endemic in Mexico and Western Africa. However, beside in humans, genotypes 3 and 4 have been detected in pigs and other animal species. Genotype 3 is distributed worldwide and genotype 4 is mostly restricted to Southeast Asia. Endemic strains found in Europe are usually of genotype 3.

The epidemiology of HEV is rather complex, and a foodborne transmission of HEV from animal products to humans is an emerging risk, especially in the European developed countries. A few research studies indicated the

following food commodities present risk factors for onset of HEV infection: pork pies, liver pate, wild boar, undercooked or raw pork, home-made sausages, meat (in general), unpasteurized milk, shellfish and ethnic foods [33]. Nevertheless, these factors were not adequately proven due to scarce data obtained from very few systematic studies. One systematic case-control study has been performed in Germany, in which eating of any offal or wild boar meat was identified as risk factor for autochthonous hepatitis E [34]. In addition, another recent small-scaled case-control study identified eating of raw pig liver sausage as a risk factor for hepatitis E in France [35]. Earlier publications from Japan indicate direct HEV transmission by eating raw or undercooked meat from wild boar or deer by detailed analysis of small outbreaks [36]. No detailed information on hepatitis E cases, including the proportion of foodborne cases, is available for the EU which is the reason why EFSA in July 2017 advised national competent authorities to commence gathering data on HEV prevalence and/or possible HEV outbreaks [37]. Despite rough estimations that approximately 2 billion people could have been exposed to HEV [38], majority of HEV cases occurred in the endemic regions in Asia, Africa and Central America, where transmission is mainly due to faecally contaminated water. Europe is not a endemic region, but sporadic hepatitis E cases have been described in France, The Netherlands, Spain, Hungary, the UK, Denmark, Norway (39), indicating an EU-wide distribution of the virus. In Germany, HEV is notifiable as of 2001 and their data indicate that a total of 40 to 220 cases (mostly non-travelers in endemic area) per year are registered, with increasing tendency (39). In France the disease is also notifiable and 218 cases have been identified in 2008. Among these cases 146 have been identified as autochthonous cases, 23 to travels and no epidemiological data was available for 49 cases [39].

Conclusion

NoV and HAV have been recognized as priority concerns in viral food-borne transmission. However, proper diagnosis of infection caused by these agents is often hindered due to sharing general symptoms with other diseases (fatigue, dehydration, nausea, vomiting, diarrhea, and some stomach cramping), failure of notification and relatively quick resolution of signs of illness. The most important role in transmission route is attributed to infected food handlers and sewage-contaminated foods. In the latter category, complex mixtures of human and animal viruses and other pathogens may be present in a single food item, causing possible genetic recombination and subsequent uncontrolled expansion of the diversity of these pathogens. Routine harmonized surveillance of viral outbreaks, and surveillance of virus occurrence in food commodities, in combination with systematic strain typing, and joint expertise from veterinary, food, and clinical microbiologists would be recommended to aid source attribution studies and identify risk prevention measures.

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