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Current Opinion in Food Science

What makes a foodborne virus: comparing coronaviruses with human noroviruses

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In order to answer the question whether coronaviruses (CoVs) can be transmitted via foods, this review made a comparison between CoVs with the most recognized foodborne virus, human noroviruses (NoVs). As a result, although CoVs indeed have shown the possibilities to remain infectious on foods and/or food packaging materials long enough (from several days to several weeks) to potentially cause transmission, they seem to be less persistent than NoVs towards common disinfection practices with alcohols, chlorine and ultraviolet (UV). More importantly, the chance of foodborne transmission of CoVs is considered low as CoVs mainly spread through the respiratory tract and there is no clear evidence showing CoVs can follow fecal-oral routes like human NoVs and other foodborne viruses.

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Current Opinion in Food Science 2021, **42**:1–7

This review comes from a themed issue on **Food safety**

Edited by **Tian Ding**

For complete overview of the section, please refer to the article collection, "Food Safety 2021"

Available online 4th May 2020

https://doi.org/10.1016/j.cofs.2020.04.011

Introduction

Human noroviruses (NoVs) are the most frequently linked virus with foodborne outbreaks, and as such are identified as the foodborne virus with the highest priority worldwide. In 2015, the World Health Organization (WHO) listed human NoVs as the 'Number 1' cause of foodborne illnesses [1]. Next to NoVs, commonly recognized foodborne viruses also include hepatitis A virus, hepatitis E virus, rotaviruses, astroviruses, and so on [2].

Because of the current pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), numerous concerns have been raised over whether SARS-CoVs-2 can be transmitted via foods and/or food packaging materials. Indeed, the possibility of foodborne transmission cannot be ruled out for any virus. However, these possibilities should be understood in-depth as supported by scientific data and analysis so that the virus spread could be controlled in a more focused and efficient way. In this review, we intend to make a comparison between coronaviruses (CoVs) with the most recognized foodborne virus, human NoVs, in order to supply evidence to evaluate the possibilities of foodborne transmission of CoVs. The comparisons were performed from four different perspectives including the epidemiological evidence, their presence in foods, their persistence in food systems, and their relevant clinical manifestations.

Foodborne illnesses are linked clearly with the consumption of contaminated foods

In 2015, WHO estimated 684 million diarrheal disease cases caused by human NoVs annually, amongst which 212 000 deaths were caused [2]. The link between many of the illnesses and the human NoVs-food contaminated-food consumption has been clearly demonstrated (Table 1) thanks to the comprehensive investigations among all the components of a food control system. Typically, a successful foodborne outbreak investigation will need collaborative efforts from food law and regulations, food control management, inspection services, epidemiological and food monitoring (laboratory services) and consumers' education of and communication. Verhoef *et al.* [3] estimated the proportion of foodborne infections caused by human NoVs on a global scale to be as high as ~14%. Meanwhile, it should be well noted that large-scale outbreaks are often the result of a combination of several transmission routes. For example, the virus can first infect a sensitive population by food, water or an asymptomatic shedder, and a more efficient viral spread in a large group of population could be followed by direct person-to-person contact or via a contaminated environment.

In comparison, to the best of our knowledge, despite the long history and wide spread of CoVs in human communities, there is no epidemiological evidence showing that any of the illnesses was due to food consumption.

Foodborne viruses are detected frequently from foods

The discovery of human NoVs from food systems is not rare. Numerous reports have been published for human NoVs screening from food and environmental samples (Table 2). The most common categories of food linked to outbreaks are shellfish, which can bio-accumulate viral particles from a large volume of water and is often consumed uncooked; and fresh produce, especially soft

Table 1

Internationally reported foodborne human NoV outbreaks in recent years

Foods involved in the outbreaks	Period and origin	Epidemiological description	Laboratory investigation	Reference
Oyster	Jan. 2020, Denmark and Sweden	At least 180 people in Denmark and 70 people in Sweden were sick with vomiting and diarrhea.	Symptomatic individuals and oyster samples were positive for NoV.	[20]
Turkey	Mar., 2018, Spain	The acute gastroenteritis outbreak affected 137 out of 361 people of a nursing home.	Ten of the 28 stool samples were positive for NoVs (two GI, six GII and two GI/GII). Turkey was suggested to be the initial source of the outbreak and was subsequently spreading via person-to-person transmission.	[21]
Mussels	2017, Spain	Thirty-nine people were sick after consuming mussels contaminated with NoV	Three stool samples from symptomatic individuals were positive for NoV. Mussel samples from the affected batch were positive for NoV GI and GII.	[22]
Oyster	Jan., 2017, New Zealand	Eleven people became ill after consuming oyster harvested from Mahurangi Harbour	NoVs identified from symptomatic individuals and oysters were the same.	[22]
Chipotle chili	Oct. and Nov., 2016, United Kingdom	A total of 1112 customers and staff reported with gastroenteritis after eating at all branches of a restaurant group	Thirty out of 48 samples from staff were positive for NoV strain GII.6. New chipotle chili imported from outside the European Union was most likely to be the vehicle of the transmission	[23]
Coleslaw	2015, Sweden	A two-episode outbreak; the first outbreak affected 542 out of 1109 employees in a large office-based location in Stockholm. Three weeks later (second outbreak), 54 employees and a restaurant personnel fell ill with gastrointestinal-symptoms.	First outbreak: 8 faecal samples from symptomatic individuals and coleslaw samples were positive for NoV GII. Nucleotide sequencing of the faecal samples reveals that the outbreak strain belongs to GII.6 genotype. Second outbreak: 3 employees and 2 out of 10 restaurant personnel were positive for NoV GII. The close connection between two outbreaks suggests the possible spread of the same NoV genotype (GII.6), which could be attributed to a mixture of foodborne and person-to-person transmission.	[24]

berry fruits and leafy green vegetables, which can be contaminated during the primary production, and are generally consumed without effective treatment to get rid of the contaminated viruses. However, one must realize that any food can be implicated in outbreaks, especially when the contamination is due to infected food handlers [1].

Again, no record over the presence of CoVs in foods could be found from the literature. One may argue that the CoV presence could have been understudied and in the future, especially with the use of metagenomics technologies, CoVs might be able to be found within the viromes of foods. However, care should be taken when interpreting the results of virus detection from foods with the use of molecular methods. In-depth understanding over the virus quantities in relation to a dose-dependent effect and the virus viability (as the molecular methods detecting the presence of nucleic acids are not able to differentiate between infectious and non-infectious viruses) are of crucial importance in order to determine the relevant public health influence.

Foodborne viruses show high stability and resistance towards environmental stress in food systems

Figure 1 illustrates the foodborne transmission routes of human NoVs. Since viruses cannot multiply themselves without a host, after being shed to the environment, the viruses must be able to resist the possible environmental stress in the food systems, such as solar irradiation, desiccation, high or low temperature, unfavourable chemicals, and so on, and remain infectious for durations long enough until being ingested again. In fact, human NoVs are known as the 'super survivor' in the food systems as shown by numerous studies (Table 3).

Table 3 intends to compare the stability between NoVs and CoVs under different possible conditions in the food systems. Since molecular methods underestimate largely the infectivity decrease of viruses, we only included data generated with the use of cell culture based methods or from human volunteer studies. Although there have been recent breakthroughs reported in human NoV tissue culture models [4•], it is not yet commonly used for

Table 2

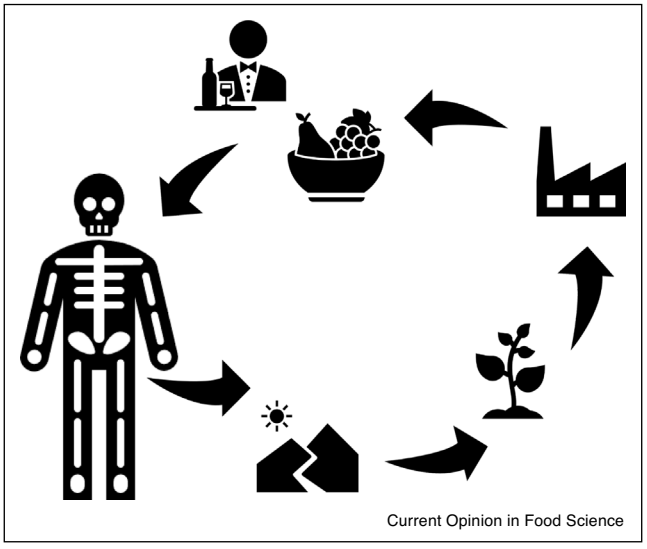
Internationally reported human NoV screenings from foods in recent years

Sample type	Period and origin	Positive rate	Detection and analysis methods	Reference
Fresh produce (raspberries and lettuce) frozen produce (Raspberries)	Mar., 2015 to Apr., 2017, the United Kingdom	Human NoV was detected in 5.3% (30/568) of lettuce samples, 2.3% (7/310) of fresh raspberry samples and 3.6% (10/274) of frozen raspberry samples.	Real-time RT-PCR (Taqman)	[25]
Fresh/frozen berries (strawberries, blueberries, raspberries, cranberries, blackberries and blackcurrants)	Jan., 2016 to Dec., 2017, China	Human NoV was detected in 9% (81/900) of frozen and 12.1% (109/900) of fresh domestic retailed berry samples.	Real-time RT-PCR (Taqman)	[26]
Fresh seafood (oysters, clams, shrimps and finfish)	India	NoV GII was detected in 41 out of 104 (41.3%) fresh seafood samples. The incidence of NoV was the highest in bivalves (52.7%–39/74), followed by finfish (16.7%–2/12) and lastly crustaceans (11%–2/18)	Reverse-transcription PCR (RT-PCR), nested PCR, Southern hybridization (for confirmation purpose)	[27]
Shellfish (oysters and clams)	Oct., 2015 to June., 2016, Vietnam	Human NoV was detected in 81.8% (99/121) of the analyzed samples. Multiple strains of NoV were identified (GI.2, GI.4, GI.5, GI.6, GII.3, GII.4, GII.6, GII.7, GII.13, GII.14, GII.17, GII.21 and GII.18)	Real-time RT-PCR (Taqman)	[28]
Shellfish (oyster)	Sep., 2015 to Sep., 2016, China	Human NoV was detected in 20.7% (135/652) of the oyster samples.	Real-time RT-PCR (Taqman)	[29]
Shellfish (oyster)	Nov., 2014 to Mar., 2015, Japan	NoV GII was detected in 89% (48/54) of the composite oyster samples pooled from 162 individual oysters. Multiple genotypes of GII were identified (GII.3, GII. 4, GII.6, GII.13, GII.17)	Reverse transcription (RT) and quantitative real time PCR (qPCR), nested PCR (for unquantifiable but possibly positive sample), pyrosequencing (for genotyping and phylogenetic analysis).	[30]

routine food and environmental testing due to the presence of residual food matrix components as well as the cost and labour implications. Surrogates including feline calicivirus (FCV), murine norovirus (MNV), and coliphage MS2 that share pathological and/or biological features with human NoVs have been widely used to study the stability of human NoVs. For CoVs, although the most-of-interest strains are SARS-CoVs and other severe syndrome strains such as Middle East respiratory syndrome coronavirus (MERS-CoV), working with BSL-3 laboratory containment places can cause significant practical challenges, and thus researchers have employed surrogates such as transmissible gastroenteritis virus (TGEV), a diarrheal pathogen of swine, and mouse hepatitis virus (MHV), a respiratory and enteric pathogen of laboratory mice, to study the survival and persistence of CoVs. Consequently, large variabilities were observed even within the NoVs or CoVs used in different studies (Table 3).

Low temperature is favourable for both NoVs and CoVs to survive both on food-contact surfaces/solid foods and in water/liquid foods as shown consistently in multiple studies as summarized in Table 3. However, the influence of relative humidity (RH) is contradictory for different viruses. HAV survived better at higher RH [5], while MS2 and MERS-CoV survived better at lower RH [5,6].

Figure 1



Foodborne transmission routes of human NoVs.

Similarly, no consensus could be reached for the influence of organic (food) matters. On one hand, MNV showed 6.2-log reduction on residue-free coupons and only 1.4-log reduction on coupons with lettuce, cabbage, or ground

Table 3

The stability of NoVs and CoVs and their surrogates under different conditions as reported in the literature

Conditions	NoVs and the surrogates		CoVs and the surrogates	
	Virus stability	Reference	Virus stability	Reference
On possible food-contact surfaces and solid foods	MNV, NoV surrogate at room temperature for 28 days: Rank order of reduction, from highest to lowest, was stainless steel (2.28-log reduction after 28 days), plastic, rubber, glass, ceramic, and wood (1.29-log after 28 days).	[31]	SARS-CoV-2 at 21–23°C and 40% relative humidity (RH): more stable on plastic (3.1-log reduction after 3 days) and stainless steel (3.1-log reduction on plastic after 2 days) than on copper and cardboard, and viable virus was detected up to 3 days after application to these surfaces.	[32•]
	On dried stainless steel surfaces for 7 days: MNV and FCV showed ~1-log reduction at 4°C; ~4-log reduction at room temperature after 7 days.	[33]	SARS-CoV remained stable on plastic surface at room temperature with 40−50% RH for up to 4 weeks, yet lost its infectivity significantly at 38°C with >95% RH during 24 hours in air.	[34]
	On stainless steel coupons for 30 days at 25°C: MNV showed 6.2-log reduction on residue-free coupons and 1.4-log reduction on coupons with lettuce, cabbage, or ground pork residues	[7]	Air-dried SARS-CoV on polystyrene surfaces retained its infectivity for 6 days at 4°C.	[35]
	Bacteriophage MS2 4°C: <1-log reduction for all produce types by day 7, <2-log reduction in cabbage and carrots by day 87; 8°C: <1-log reduction for all produce types by day 7, ~1-log reduction in tomato, cabbage, carrots and lettuce by day 39; 22°C: 1-log reduction on lettuce and <1-log reduction on tomato and parsley by day 7	[36]	MERS-CoV survived on both plastic and steel surfaces surfaces after 48 hours at 20°C, 40% RH, while it remained viable only for 8 hours at 30°C, 80%RH and 24hours at 30°C, 30% RH. At 20°C, MERS-CoV's viability decreased 7% at 40% RH, and 89% at 70% RH respectively.	[6]
	Hepatitis A virus (HAV), MS2, MNV on oyster and peppers at 4°C, 15°C, 25°C, and 40°C: viruses survived best at 4°C and were inactivated most at 40°C. On oysters, a 1-log reduction of both HAV and MNV occurred at 4°C, even after 14 days. However, a 5-log reduction of MNV occurred on peppers at 4°C. MNV showed the shortest survival duration on peppers at all temperatures compared to the other viruses. Viral survival was better on oysters than on peppers. At a given temperature, HAV survived better at higher RH, while MS2 survived better at lower RH. At 40°C, inactivation of HAV was1 log at 50% RH but only 0.1-log at 70% RH on day-1 postinoculation.	[5]	Human coronavirus (HCoV) strain 229E survived on lettuce during 2 days of storage at 4°C, yet became non-infectious by day 4 (reduction > 1.31-log). No HCoV could be recovered from raspberries or strawberries after spiking.	[37]
In water and liquid foods	MNV showed infectivity reduction rate of 0.16-log PFU/day in surface water and 0.04-log PFU/day in groundwater at 25°C.	[8]	HCoV survived (with 99.9% decrease of infectivity) for 10 days at 23°C, for >100 days at 4°C in tap water, yet for only 2–4 days in wastewater.	[9]
	Norwalk virus (NV, prototype of NoVs) remained infectious at least for 61 days in groundwater at room temperature in the dark as tested by human volunteer studies.	[38]	At 25°C, transmissible gastroenteritis (TGEV) survived for 22 days, and mouse hepatitis virus (MHV) survived for 17 days in reagent-grade water, whereas in wastewater, TGEV survived for 9 days and MHV survived for 7 days (with 99% decrease of infectivity). At 4°C, both viruses survived longer than four weeks.	[10]
	MNV showed no reduction, FCV showed 3-log PFU/mL reduction, MS2 showed <1-log reduction in milk after 21 days at refrigeration (4°C).	[39]	MERS-CoV survived in dromedary camel milk at 4°C for 72 hours (with 37% reduction of infectivity), while it lost infectivity rapidly at 22°C in dromedary camel milk, goat milk, and cow milk (88%-99% reduction) within 48 hours of storage.	[40]
Towards alcohols	Regardless of concentration or exposure time, alcohols slightly reduced, but did not completely inactivate, human norovirus (3 GII.4 strains tested by the enteroid culture model).	[11•]	No SARS-CoV residual infectivity was detected after fixation with 70% ethanol for 10 min or 100% ethanol for 5 min. Isopropanol 70% and 100% achieved >3.31-log reduction of SARS-CoV infectivity after 30 s.	[35]
Towards chlorine	Complete inactivation of the 3 GII.4 viruses occurred at concentrations at 50 ppm of chlorine after incubating the solutions for 1 min at room temperature strains tested by the enteroid culture model.	[11•]	SARS-CoV could be completely inactivated with 10 ppm chlorine for 10 min or more, and with 20 ppm chlorine for 1 min or more.	[41]
Towards UV	The susceptibility of MHV was 7-10 times that of the MS2.			[12•]

pork residues [7]. On the other hand, MNV showed infectivity reduction rate of 0.16-log PFU/day in surface water and 0.04-log PFU/day in groundwater at 25°C [8]. Similarly, human coronavirus (HCoV) survived for 10–100 days in tap water, yet for only 2–4 days in wastewater [9]. At 25°C, TGEV survived for 22 days, and MHV survived for 17 days in reagent-grade water, whereas in wastewater, TGEV survived for 9 days and MHV survived for 7 days [10]. Therefore, the matrices with different components tested with the viruses may play different roles, either in viral protection from the environmental stress, or as anti-viral agents alone or together with the external stress.

In addition, different experimental set-ups were used in different studies, including the tested environmental parameters, the virus spike levels, the test durations, the virus recovery methods, and the data interpretation methods (log-reductions versus durations until the viruses became non-infectious), and so on. Therefore, it is not possible to make direct comparisons between NoVs and CoVs over their stabilities on foods (both solid and liquid foods) or possible food-contact surfaces. Nevertheless, it seems that both NoVs and CoVs were able to remain infectious on foods and/or food packaging materials long enough (from several days to several weeks) to potentially cause transmission especially at low temperatures.

Fortunately, when it comes to the disinfection studies, more straightforward comparisons become possible thanks to the availability of studies evaluating human NoV viability with the use of tissue culture model [11•] and studies directly comparing NoV and CoV surrogates [12•]. According to the results demonstrated in Table 3, NoVs were clearly much more resistant than CoVs towards alcohols, chlorine and ultraviolet (UV) disinfection.

Foodborne viruses are transmitted via fecal-oral routes

So far, all of the well-recognized foodborne viruses are transmitted via fecal-oral routes. For human NoVs, although our understanding on the cellular pathways that control infection and the exact pathogenesis remains limited [13,14], the recent breakthrough of human NoV *in vitro* cultivation systems with mucosa-derived intestinal epithelial organoids [4•] reveals clearly that human NoVs infection occurs primarily in the human digestive tracts. Besides, the clinical manifestations of human NoVs also have the following features being believed to contribute to its 'achievement as a successful foodborne virus'. First, human NoVs are extremely contagious. The infectious dose of human NoVs was estimated to be as low as 10 particles [15]. Second, once infected, human NoV particles can be shed from the stool and vomit of the patients in large quantities (e.g. up to $>10^{10}$ genomic copies per gram of feces [16]). Moreover, it has been discovered that asymptomatic infections with long-term fecal shedding (up to three weeks) of human NoVs can have a high prevalence, especially in the group of children [17].

CoVs, including the newly emerged SARS-CoV-2, mainly spread through the respiratory tract. There are indeed reports showing the presence of CoVs in human fecal samples. For instance, in a recent investigation, 41 (55%) out of 74 SARS-CoV-2 infected patients were tested positive for SARS-CoV-2 RNA in their fecal samples and the fecal shedding remained for a mean of 27.9 days after the first symptom onset [18]. However, there is so far no clear evidence showing SARS-CoV-2 can cause infection in human digestive tracts. Considering the high probability of infection of SARS-CoV-2 (basic reproduction number estimated to be above 2.0 by WHO [19]), assumptions could be made that the viruses may migrate from the oral ingestion to the respiratory tracts via, for instance, the throat. However, this assumption will need sound experimental and/or clinical supports not only in a qualitative way (to show whether it is possible for the virus to migrate from oral ingestion to the respiratory tract), but also in a quantitative way. Since if large quantities of viruses must be ingested in order to cause the migration, the chance of such occurrence could be very low in reality.

Conclusions

On the basis of our understanding, four important features are shared by foodborne viruses. I) Clear epidemiological evidence showing the link between relevant illnesses and the consumption of virus-contaminated foods; II) Records of virus presence in foods by the monitoring or surveillance studies, which in reality facilitate due diligence in the food supply chains or initiate recalls; III) High stability and resistance towards environmental stress in the food systems; IV) Fecal-oral transmission routes with infection occurring primarily in the human digestive tracts.

In correspondence, the chance of foodborne transmission of CoVs is considered low and thus CoVs should not be recognized as foodborne viruses. CoV infection has never been found to link with food consumption, and so far CoVs have never been detected from foods either. Although CoVs indeed showed the possibilities to remain infectious on foods and/or food packaging materials long enough (from several days to several weeks) to potentially cause transmission, they were found to be less resistant to chemical and physical disinfections than NoVs. More importantly, CoVs mainly spread through the respiratory tract and there is no clear evidence showing CoVs can follow fecal-oral routes and cause infection in the human digestive tracts.

In the future, the possibility of CoV infection via oral ingestion should be monitored closely, as many facts of

these viruses still remain unrevealed and the viruses may evolve rapidly. In addition, care should be taken when interpreting results obtained with the emerging molecular technologies. As the trace of virus genetic materials may neither necessarily represent the presence of viable viruses thus nor the public health threats. Lastly, when multiple transmission routes are identifiable, comprehensive consideration is necessary to set up the priorities and to control the virus spread efficiently.

Conflict of interest statement

Nothing declared.

Acknowledgement

This study was supported by a Ministry of Education (MOE) academic research fund (AcRF) TIER 1 project 'Study of important foodborne viruses from relevant foods in Singapore' (Jan 2019 to Dec 2021).

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